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Nitrification in a zeoponic substrate

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Abstract

Clinoptilolite is a zeolite mineral with high cation exchange capacity used in zeoponic substrates that have been proposed as a solid medium for growing plants or as a fertilizer material. The kinetics of nitrification has not been measured for NH_4^+ saturated zeoponic substrate. Experiments were conducted to evaluate the production of NO_2^- and NO_3^-, and nitrifier populations in zeoponic substrates. Small columns were filled with zeoponic substrate inoculated with a commercial inoculum or soil enrichment culture of nitrifying bacteria. In addition to column studies, a growth chamber study was conducted to evaluate the kinetics of nitrification in zeoponic substrates used to grow radishes (*Raphanus sativus* L.). The zeoponic substrate provided a readily available source of NH_4^+, and nitrifying bacteria were active in the substrate. Ammonium oxidation rates in column studies ranged from 5 to 10 μg N g^{-1} substrate h^{-1}, and NO_2^- oxidation rates were 2 to 9.5 μg N g^{-1} substrate h^{-1}. Rates determined from the growth chamber study were approximately 1.2 μg N g^{-1} substrate h^{-1}. Quantities of NH_4^+ oxidized to NO_2^- and NO_3^- in inoculated zeoponic substrate were in excess of plant up-take. Acidification as a result of NH_4^+ oxidation resulted in a pH decline, and the zeoponic substrate showed limited buffering capacity.

Introduction

Zeoponic plant growth substrates have been developed to grow plants during long-term space missions (Ming, 1989). Zeolites are hydrated aluminosilicates with extra framework alkali and alkaline earth cations that have the capability to exchange some of their constituent cations without change of structure (Ming and Mumpton, 1989). A primary benefit of using a zeoponic substrate is that sufficient nitrogen in the form of NH_4^+ may be available on cation exchange sites for multiple croppings.

Some evidence suggests that a balance between NH_4^+ and NO_3^- is desirable in promoting plant growth and seed development. Plants may utilize NH_4^+ or NO_3^- ions effectively as a source of nitrogen (Maynard and Barker, 1969). However, species differ in their ability to absorb or assimilate different nitrogen sources (McKee, 1962). Ammonium, as a sole source of nitrogen, was deleterious to the growth of radish plants (Goyal et al., 1982; Weir et al., 1972), and many other higher plants (Findenegg, 1987; Hoff et al., 1974).

Understanding the nitrogen dynamics in zeoponic systems is critical to ensure efficacy of this substrate as a plant growth medium. In previous research, nitrifying bacteria have been added to zeoponic substrates so that some NO_3^- may be formed to provide balanced nitrogen nutrition; however, the kinetics of nitrification were not measured (Steinberg et al., 2000).

Nitrate can be provided to a plant growth system via nitrification, which involves the oxidation of NH_4^+ to NO_2^- and then to NO_3^-. It is carried out exclusively by microbiological agents (Bohlool et al., 1977). Different microbial populations carry out NH_4^+ oxidation and NO_2^- oxidation. In soil systems, these successive steps are chiefly carried out advanced life support systems during long-term space

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missions. Knowledge of nitrification activities is essential to maintaining the proper balance of inorganic nitrogen forms. Too much nitrification may result in toxicity to plants or humans consuming plants.

Materials and methods

Three experiments were conducted. Experiment I utilized a zeoponic mixture in small plastic columns to determine kinetics of nitrification using a commercially available inoculum. Experiment II was conducted in the same fashion but soil was used as the inoculum. Experiment III was conducted at Johnson Space Center, Houston, Texas under conditions used to grow plants in zeoponics.

Experiment I

Zeoponic mixture

The zeoponic substrate was similar to that described by Steinberg et al. (2000), and was basically composed of NH_4^+- and K^+-saturated clinoptilolitic tuff, dolomite, and synthetic nutrient-substituted hydroxyapatite. The substrate was washed thoroughly with distilled water to remove NO_3^- present from processes used in development of the zeoponic substrate.

Inoculum

A commercial inoculum of nitrifying bacteria (Turbo Start 700, Fritz-Zyme, Mesquite, Texas) included both NH_4^+ and NO_2^- oxidizing bacteria. The commercial inoculum of nitrifying bacteria contained a concentration of 1×10^7 cells mL^{-1} NH_4^+ oxidizing bacteria and 1×10^8 cells mL^{-1} of NO_2^- oxidizing bacteria based on most probable number (MPN) enumerations by the method of Schmidt and Belser (1994). The MPN method may result in low estimates of nitrifier populations (Schmidt and Belser, 1994).

Treatments

Small columns, 10 cm in length and 1.5 cm in diameter, were filled with 10 g of zeoponic substrate and inoculated with 5-mL of diluted commercial inoculum. The treatments were set-up in triplicate and were as follows: no inoculum, an inoculum containing 1.0×10^2 NH_4^+ oxidizing bacteria g^{-1} substrate and 3.1×10^3 NO_2^- oxidizing bacteria g^{-1} substrate denoted as low inoculum, and an inoculum containing 1.0×10^4 NH_4^+ oxidizing bacteria g^{-1}substrate and 3.1×10^5 NO_2^- oxidizing bacteria g^{-1}substrate denoted as high inoculum. Numbers of nitrifying bacteria were determined by the MPN method on zeoponic medium within an hour of inoculation and due to limitations of the MPN method the numbers may have been underestimated (Schmidt and Belser, 1994).

Measurements

Columns were leached daily with 10 mL distilled water for 1 month and the leachate collected. The leachate was analyzed for NO_2^--N and NO_3^--N using the copper-cadmium reduction method described by Bundy and Meisinger (1994). The pH of leachate was determined with a pH electrode and digital ionalyzer. Data are presented as cumulative μg N, either NO_2^- or NO_3^-, g^{-1} zeoponic substrate and was the product of 10 mL leachate and concentration of nitrogen form divided by 10 g zeoponic substrate.

Experiment II

Inoculum

Ships clayey soil (very fine, mixed, thermic Chromic Udic Haplusterts), was collected from the surface horizon (0–15 cm) of a field near College Station, Texas. It was air dried at room temperature and ground to pass a 2-mm sieve. To stimulate the growth of nitrifying bacteria the soil was re-wetted to field capacity, 100 μg NH_4^+-N g^{-1} soil was added and the sample was incubation for 1 month at room temperature. At the end of incubation, an active population of nitrifying bacteria was confirmed using the short-term nitrification rate assay (Schmidt and Belser, 1994).

Treatments

Before inoculation and putting substrate into the columns, 100 g of substrate was shaken by hand with 200-mL distilled water to remove background NO_3^- that was present. The liquid fraction was decanted and wash was repeated with another 200 mL distilled water to remove residual NO_3^-. The washed zeoponic substrate was air-dried overnight. The treatments were set-up in triplicate. One treatment was 0.1 g of soil enriched for nitrifying bacteria mixed with 10 g of zeoponic substrate. This provided 1.1 x 10^4 NH_4^+ oxidizing bacteria g^{-1} zeoponic substrate and 4.9×10^3 NO_2^- oxidizing bacteria g^{-1} zeoponic substrate. The second treatment was 0.1 g sterile soil mixed with 10 g of zeoponic substrate. The soil that had been enriched for nitrifying bacteria was subjected to auto-

claving (121 °C at 0.10 MPa for 15 min) for a source of sterilized soil.

Measurements

Columns were leached daily with 10 mL of distilled water, for a 1-month period, and the leachate was analyzed for NO_2^--N, NO_3^--N, and pH. Data are presented as for Experiment I.

Experiment III

Inoculum

A commercial inoculum of nitrifying bacteria (Nitrotreat, Enviroflow, Inc., Manassas, VA) included both NH_4^+ and NO_2^- oxidizing bacteria.

Treatments

A pot experiment with Cherry Belle radishes was conducted in a plant growth chamber at NASA Johnson Space Center. Each pot was filled with 450 g of the zeoponic mixture, described for Experiment I. Treatments included pots with commercial inoculum and no plants, and pots with commercial inoculum and plants. The number of nitrifiers in the inoculum was not determined. There was also a control treatment that received no inoculum, and no plants. Pots were arranged in a completely randomized design with three replicates per treatment, and two replicates for controls.

Plant Growth

Seven radish seeds were sown in each pot, and 1 week after emergence thinned to three seedlings. The environment was maintained at 23 °C and 75% relative humidity under a 16-hr photoperiod with a light intensity of 300 μmol m^{-2} s^{-1}. Throughout the growth period, the soil moisture content was kept at field capacity via an irrigation system and there was minimal leaching, but all leachate was collected.

At the beginning and at weekly intervals, the pots were leached with three batches of 400 mL distilled water. Leachates were collected and analyzed for NO_2^- and NO_3^- using the copper-cadmium reduction method (Bundy and Meisinger, 1994), and pH was measured. The pH of the leachate was determined with a pH electrode and pH was measured with a digital ionalyzer.

Short-term nitrification assay

Plugs of the zeoponic substrate, approximately 10 g, were removed weekly from the pots to determine activity of nitrifying bacteria using the short-term nitrification assay (Schmidt and Belser, 1994). One gram of the sample was used in the assays. To remove NO_2^- and NO_3^- in the sample, it was washed by vortexing with 10 mL of distilled water in a test tube. The liquid fraction was decanted and passed through a 0.45-μm filter on a vacuum filtration apparatus. The process was repeated, and then 20 mL of distilled water were used to wash the substrate from the test tube into the filtration apparatus. The filter and residue on the filter were then placed into a 150 mL Erlenmeyer flask with 10 mL of NH_4^+-oxidizer media for the short-term nitrification assay as described by Schmidt and Belser (1994). At time zero, samples were analyzed for NO_2^- and NO_3^- (Bundy and Meisenger, 1994). Incubations were for 2-h at 20 °C on a rotary incubator shaker. Following the 2-h incubation, solution was removed from the flask, and analyzed for NO_2^- and NO_3^-.

Radish harvest

Twenty-one days after planting, radishes were harvested. Shoots and roots were removed from the pot by carefully washing substrate away from the plant material with a gentle stream of water. The plant material was oven dried at 65 °C for 24 h before determining the dry weight. The material was then ground to pass through a 30 mesh screen. The ground material was analyzed for total nitrogen (Sheldrick, 1986), and NO_2^- and NO_3^- (Keeney and Nelson, 1982).

Uninoculated columns maintained a pH above 7.4 (Figure 4). The pH for treatments inoculated with the high inoculum declined to 5.8 and the pH of the low inoculum treatment declined to 6.2 during the study (Figure 4).

Leachate from zeoponic substrate amended with sterilized soil was approximately pH 8 over the course of the study (Figure 8). Leachate from treatments amended with soil enriched with nitrifying bacteria declined to pH 6.6 by the end of the study (Figure 8).

Experiment III

There were no statistically significant differences for short term nitrification rates between treatments having radish plants and those not having radish plants 1, 2, and 3 weeks after planting (Table 1). Ammonium

Table 1. Nitrification activity of zeoponic substrate collected as core samples at 1, 2, and 3 weeks from pots growing plants and from pots not growing plants*

Treatment	Parameter	μg N g^{-1} zeoponic substrate h^{-1}		
		Week 1	Week 2	Week 3
No plant	NH_4^+ Oxidation	1.10 ± 0.19	1.78 ± 0.15	1.73 ± 0.37
Plant	NH_4^+ Oxidation	1.21 ± 0.14	1.49 ± 0.30	1.78 ± 0.20
No plant	NO_2^- Oxidation	0.40 ± 0.15	0.61 ± 0.23	0.75 ± 0.20
Plant	NO_2^- Oxidation	0.38 ± 0.04	0.70 ± 0.18	0.74 ± 0.13

*No statistically significant differences between having or not having plants present.

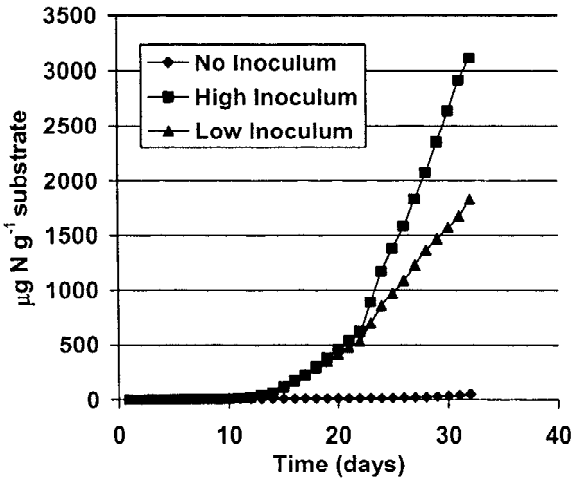

Figure 1. Gross NH_4^+ oxidation based on cumulative NO_2^- -N and NO_3^--N leached from columns containing zeoponic substrate receiving two rates of inoculation, and an uninoculated control.

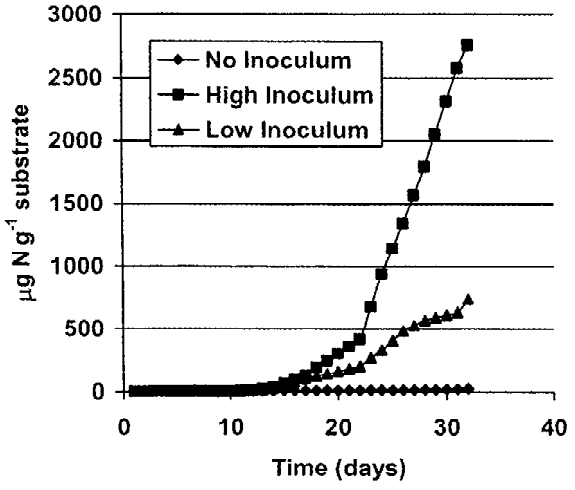

Figure 2. Nitrite-oxidation based on cumulative NO_3^--N leached from columns containing zeoponic substrate receiving two rates of inoculation, and an uninoculated control.

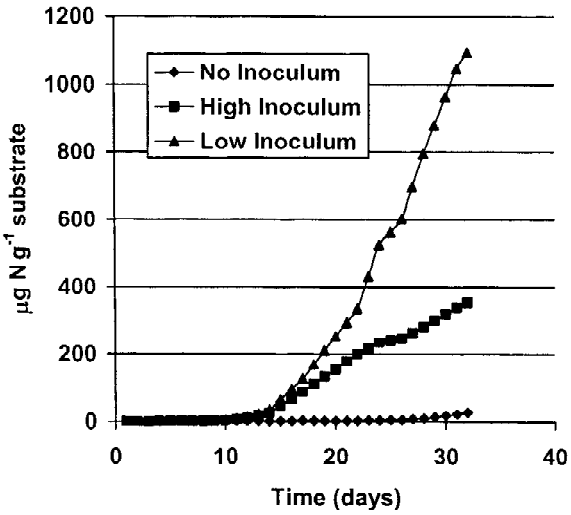

Figure 3. Cumulative $N)_2^-$–N leached from columns containing zeoponic substrates receiveing two rates of inoculation, and an unioculated control.

oxidation increased from the first week of study to the second week. Nitrite oxidation over the course of the study showed lower rates of activity when compared to NH_4^+ oxidation (Table 1). Rates of NO_2^- oxidation increased from week 1 to weeks 2 and 3.

Considerable amounts of NO_2^- and NO_3^- were leached from pots in the growth chamber study. The NO_3^- collected in the leachate over the 3-week growth period was approximately 72.7 + 12.7 mg NO_3^--N in the treatments with no plants and 117 + 9.13 mg NO_3^--N in treatments with plants. The NO_2^- collected in the leachate was 316 + 37.0 mg NO_2^--N in treatments with no plants and 288 + 29.3 mg NO_2^--N in treatments with plants.

The shoot portion of the radish plant contained the highest concentration of NO_3^- on a dry weight basis (Table 2). The radish (edible portion of plant) con-

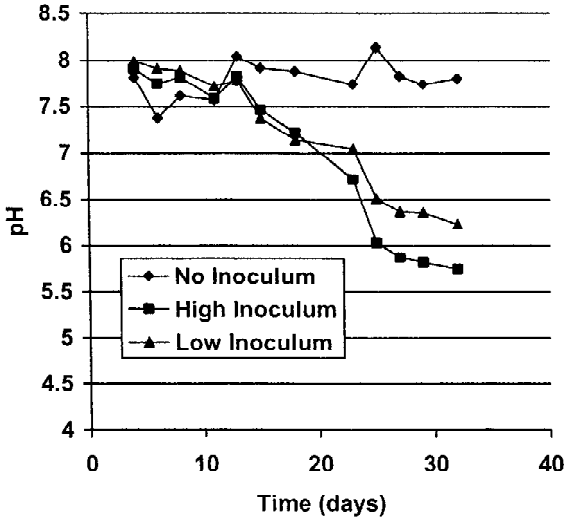

Figure 4. pH values of the leachate collected from columns containing zeoponic substrate over the one-month incubation period.

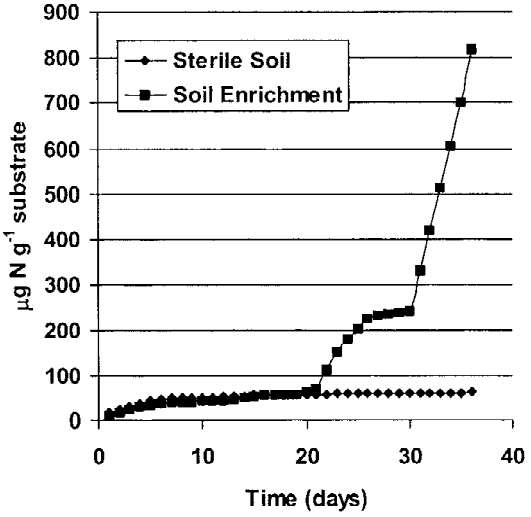

Figure 6. Nitrite-oxidation based on cumulative NO_3^--N leached from columns containing zeoponic substrate inoculated with soil enriched for nitrifing bacteria, and an uninoculated control.

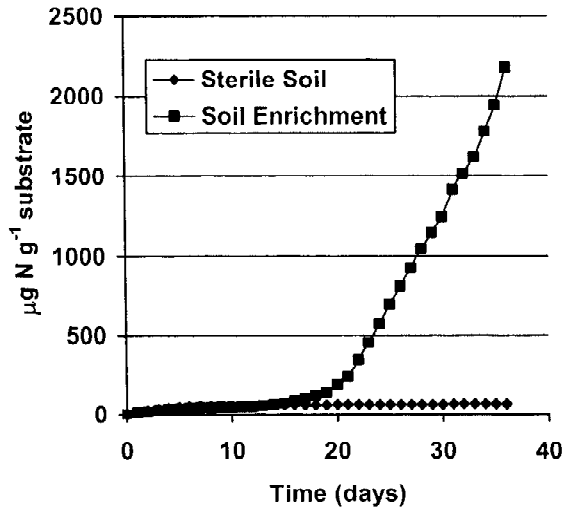

Figure 5. Gross NH_4^+ oxidation based on cumulative NO_2^--N and NO_3^--N leached from columns containing zeoponic substrate inoculated with soil enriched for nitrifying bacteria, and an uninoculated control.

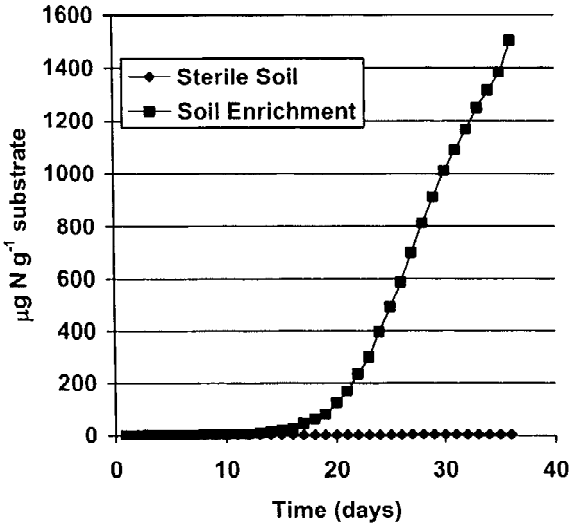

Figure 7. Cumulative NO_2^-–N leached from columns containing zeoponic substrate inoculated with soil enriched for nitrifing bacteria, and an uninoculated control.

tained a lower concentration of NO_3^-, but the highest concentration of NO_2^-. The concentration of NO_3^- in the plant material was much higher than the concentration of NO_2^-. The percentage of total N present as NO_3^- was less than 20% and was less than 0.06% for NO_2^-.

Leachate from pots receiving no commercial inoculum of nitrifying bacteria maintained an alkaline pH for 3 weeks (Table 3). The pH in leachate from pots inoculated with nitrifying bacteria declined to 6.95 at 3 weeks of incubation (Table 3).

Discussion

Nitrification was very active in the zeoponic substrate for both commercial inoculum sources (Figures 1 and 2, Table 1) and the soil enrichment inoculum (Figures 5 and 6). The results indicated three areas of

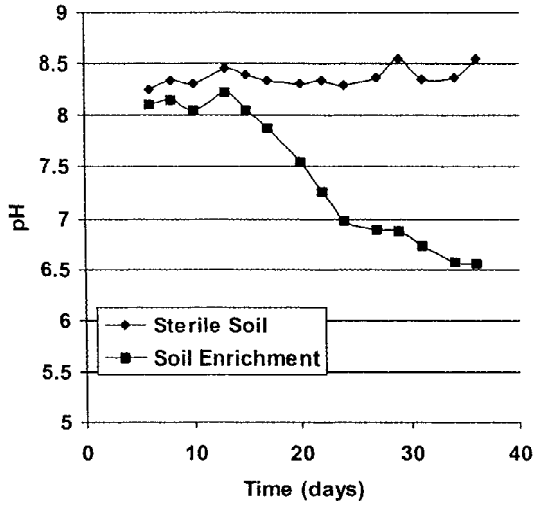

Figure 8. pH values of the leachate collected from columns containing zeoponic substrate over the one-month incubation period.

Table 2. Dry matter yield and nitrogen composition of radish plants grown in zeoponic substrate inoculated with nitrifying bacteria

	Dry weight (g plant^{-1})	Total N (g N kg^{-1})	NO_2^- (mg N kg^{-1})	NO_3^- (g N kg^{-1})
Shoot	0.39	73 ± 17 a*	0.33 ± 0.25 a	13 ± 8.6 a
Radish	0.19	36 ± 5.8 b	22.5 ± 14.5 b	4.2 ± 2.0 b
Root	0.05	43 ± 5.6 b	6.5 ± 10.4 ab	3.4 ± 0.7 b

*Means followed by the same lower case letter in a column are not significantly different (LSD, $P = 0.05$; $n = 3$).

Table 3. pH values of leachates during the 3-week period of radish growth

Treatment	Initial	Week 1	Week 2	Week 3
No Plant	7.63	6.86	7.29	6.96
Plant	7.69	7.13	7.27	6.95
Uninoculated	7.50	8.15	8.05	7.84

concern with using nitrification to provide a balance of NH_4^+ and NO_3^- for plant growth in NH_4^+ saturated zeoponic substrate. The rate of nitrification was so rapid that excessive amounts of NH_4^+ oxidized, NO_2^- accumulated, and the pH of the leachates were considerably reduced.

The rate of nitrification was high in column experiments and exceeded the rates for soils amended with NH_4^+. The rates of NH_4^+ and NO_2^- oxidation were approximately 10 μg N g^{-1} h^{-1} for the higher rate commercial inoculum (Figures 1 and 2). Initially, the number of cells used were in the low range for soils but during the incubation numbers increased to 5×10^7 NH_4^+ oxidizing bacteria g^{-1} and 8×10^7 NO_2^- oxidizing bacteria g^{-1} which would be considered high for soils (Schmidt, 1982). The population of nitrifying bacteria has been suggested as an important factor affecting the amount of nitrification occurring in soils (Frederick, 1957; Sabey et al., 1959). Belser and Mays (1982) reported NH_4^+ oxidation rates of approximately 1.3 μg g^{-1} soil h^{-1} in New Zealand soils, which contained 1×10^4 NH_4^+ oxidizing bacteria g^{-1}. Sarathachandra (1978) measured rates as high as 3.3 μg g^{-1} h^{-1} in soils with 3.6×10^5 NH_4^+ oxidizing bacteria g^{-1}and 7.7×10^6 NO_2^- oxidizing bacteria g^{-1}.

Nitrification rates necessary to produce the NO_2^- and NO_3^- in leachate collected over the plant growth period were approximately 1.7 μg N g^{-1} substrate h^{-1}. Rates determined by the short-term assay were comparable, approximately 1.2 μg N g^{-1} substrate h^{-1}(Table 1). In addition, short-term nitrification rates in the growth chamber study were considerably lower than nitrification rates for the column studies. Differences in these rates may be related to the length of the studies. The growth chamber study was conducted for only 21 days and most rapid rates of nitrification in column studies occurred after 20 days (Figures 1, 2, 5 and 6).

Inoculating with nitrifying bacteria into zeoponic substrates resulted in NO_2^- accumulation regardless of the inoculum source. Nitrite can be toxic to seed germination and plant growth (Samater et al., 1998). The soil NO_2^- level responsible for toxicity to plants ranges from as little as 2 to 100 mg NO_2-N kg soil^{-1} (Olson and Kurtz, 1982). Nitrite becomes more toxic as the pH of the nutrient media decreases, and if NH_4^+ rather than NO_3^- is the primary source of available nitrogen (Phipps and Cornforth, 1970). Bancroft et al. (1979) reported that at pH 7 plants can tolerate up to 200 mg NO_2-N kg^{-1} but at pH 4 the tolerance limit is 2 mg NO_2-N kg^{-1}. The NO_2^- from the leachate collected during the 3 weeks of our radish experiment resulted in approximately 700 mg NO_2-N kg^{-1} zeoponic substrate being collected over the 3-week study. The weekly leachings may have prevented toxic concentrations of NO_2^- from building up and affecting plant growth.

Differences in rates of NH_4^+ and NO_2^- oxidation, and perhaps population sizes of nitrifying bacteria

may explain the observed accumulation of NO_2^-. The low inoculum treatment exhibited greater accumulation of NO_2^- when compared to the high inoculum treatment (Figure 3). Apparently, the toxicity of high NH_4^+ content was relatively more detrimental to reduced numbers of NO_2^- oxidizing bacteria. This is evident from MPN counts of nitrifying bacteria. In the low inoculum treatment, there were initially 4.5 $\times$ 10^3 NO_2^- oxidizing bacteria g^{-1} and at the end of the study population size was approximately 7.8 $\times$ 10^3. When compared to the high inoculum treatment, populations increased from 3.1 $\times$ 10^5 to 7.8 $\times$ 10^7 NO_2^- oxidizing bacteria g^{-1}.

Variations in populations of nitrifying bacteria also resulted in NO_2^- accumulation in zeoponic substrate inoculated with a soil inoculum. Initially, NH_4^+ oxidizing bacteria were 10-fold higher than NO_2^- oxidizing bacteria. At the conclusion of the study, there were 2.9 $\times$ $10^7 NH_4^+$ oxidizing bacteria and 7.5 $\times$ 10^5 NO_2^- oxidizing bacteria g^{-1}. The higher initial population size of NH_4^+ oxidizing bacteria along with their lack of sensitivity to high NH_4^+ concentrations and high pH promoted NH_4^+ oxidation compared to activity of the NO_2^- oxidizing bacteria, which had lower populations, and more sensitivity to high NH_4^+ at alkaline pH.

The zeoponic substrate consisted of NH_4^+ saturated clinoptilolite with an exchange capacity of 210 cmol kg^{-1} and a pH of 7.8. Stojanovic and Alexander (1958) reported that NH_4^+-N in high concentrations caused the accumulation of NO_2^- in soils due to effects of NH_3 on NO_2^- oxidation. Morrill and Dawson (1967) concluded that an inhibitory effect of NH_3 on NO_2^- oxidizing bacteria gave rise to NO_2^- accumulation in soils and was evident primarily during the first few days.

Nitrification results in acidification of the growth environment (Darusman et al., 1991). The leachate from the zeoponic substrate, which contained dolomite to act as a pH buffer, became acidic (Figure 4). Inability of the zeoponic substrate to buffer pH during nitrification may become problematic for plant growth if the same substrate is used repeatedly and the pH of the substrate begins to match that of the leachate.

The nitrogen composition of the radish plant tissue contained a considerable amount of NO_3^- but not much NO_2^-(Table 2). According to Cantliffe and Phatak (1974), radish plants are known to accumulate NO_3^- when grown with elevated concentrations of NO_3^- in the soil. The quantities accumulated would not likely be detrimental to plant growth based on findings of Samater et al. (1998). Some research suggests that ingested NO_3^- or NO_2^- may be detrimental to human health (Comly, 1945; Correa et al., 1990; Oshima et al., 1981; Rademacher et al., 1992). Concentrations of NO_3^- or NO_2^- in plant tissue were not high enough to be of large concern since radish consumption would be limited and toxicity is not high (Corre and Breimer, 1979). However, NO_2^- and NO_3^- concentrations in the plant might have been higher without weekly leachings of substrate, which removed excess NO_2^- and NO_3^-.

Based on the dry matter yield and nitrogen composition of radish plants, approximately 37 mg total N was taken up by the plant (Table 2). Since much larger amounts of NO_3^- and NO_2^- were collected in the leachate, nitrification exceeded radish plant needs. The significance of so much NO_2^- and NO_3^- leached relative to total plant up-take indicates a need for nitrogen nutrient management. Perhaps if a higher nitrogen demand crop, like wheat, were grown there would not have been excess NO_3^-. According to Allen and Ming (1995), the plant nitrogen content in winter wheat grown in zeoponic substrates was approximately 4.6% on a dry weight basis. Based on a dry weight of 17 g pot^{-1} and a 90-day growth cycle, approximately 0.09 mg N g^{-1} substrate day^{-1} was taken up by the plant. The combined NO_2^- and NO_3^- collected in the leachate over a 3-week growth period was approximately 405 mg NO_2^- and NO_3^-, thus nitrogen available for daily plant up-take was approximately 0.04 mg N g^{-1} substrate day^{-1}.

Based on the exchange capacity of NH_4^+ from zeoponic substrates (210 cmol kg^{-1}) and the amount of NO_2^- and NO_3^- collected from the column leachings, approximately 1–2% of the NH_4^+ available on clinoptilolite exchange sites was utilized. In the growth chamber study, nitrogen lost via leaching and plant uptake (450 mg N pot^{-1}) accounted for approximately 3% of the NH_4^+ available on cation exchange sites.

Conclusions

Nitrifying bacteria were active in the zeoponic substrate. Nitrification rates exceeded those reported in soil systems, and provided excessive amounts of NO_3^- for plant uptake. Nitrite accumulation may have been due to elevated NH_4^+ concentrations in zeoponic substrates and to higher populations of NH_4^+ oxidizing bacteria than NO_2^- oxidizing bacteria. Acidi-

fication as a result of NH_4^+ oxidation resulted in a pH decline, and the zeoponic substrate showed limited buffering capacity. Based on these findings, it may be advisable to limit the use of nitrifying bacteria or inhibit nitrification in zeoponic substrates used in advanced life-support systems.

Acknowledgements

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